On the Effect of Carbon Dioxide on Geotropic Curvature of the Roots of Pisum sativum L.

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Introductory.

Many physiologists have shown that, in general, carbon dioxide exercises a narcotic or toxic influence on vegetable protoplasm, temporarily or permanently affecting its activity, according to the partial pressure under which the gas acts. De Saussure (1), as long ago as 1804, stated that, in an atmosphere containing 8 per cent. carbon dioxide, the growth of peas was less than in air; Böhm (2), in 1873, found that roots of *Phaseolus multiflorus*, after 17 days' exposure, exhibited successively less elongation in partial pressures of 2, 5, 10, and 14 per cent. carbon dioxide respectively, the temperature ranging between 17° and 19° C.; in each percentage named the growth was progressively less than in normal air. Montemartini (3), in 1892, working with roots of *Pisum*, found 7 per cent. and upwards to depress growth-activity. Chapin (4), in 1902, found the growth of roots of *Pisum sativum* and *Vicia sativa* to be diminished by 5 per cent., and arrested by 25 to 30 per cent. and upwards. Growth of the stem in the same plants was diminished by 15 per cent., and completely inhibited by 22 to 25 per cent. Experiments conducted by one of us, in conjunction with Professor Farmer, have proved that seedling peas may be kept in an atmosphere containing 20 per cent. carbon dioxide for 14 days without losing the power of renewed growth when placed in air. It is interesting to note that, in many of these plants, the plumule was destroyed, although the main root continued to grow, growth being carried on by shoots arising in the axils of the cotyledons.

Brown and Escombe (5) grew plants in increased partial pressures of carbon dioxide. The anatomy of these plants was investigated by Farmer and Chandler (6), who found the growth of the aërial parts to be diminished, while root-growth was apparently unaltered. Ewart (7) observed that carbon dioxide stops protoplasmic streaming, but he does not state the percentage employed in his experiments.

Professor J. B. Farmer and Dr. A. D. Waller, as the result of a series of experiments on the action of various substances on protoplasmic streaming in *Elodea* and *Chara*, observed that, after treatment with carbon dioxide for a

short period, the subsequent rate of streaming was temporarily increased. Montemartini had, in 1892, observed that the growth in length of pea-roots was more rapid in 4 per cent. carbon dioxide than in air, and than in percentages greater than 7, but the significance of this does not appear to have been very generally appreciated. Chapin (4), in 1902, determined the optimum percentage of carbon dioxide for the growth of higher plants to be 1 to 2 per cent., and stated that, in small quantities, the effect is stimulative, whereas in large doses it acts as a poison.

Brown and Escombe's results have shown that the utilisation of carbon dioxide is, within limits, proportional to its partial pressure. In view of the facts that the weight of the plant does not increase in proportion to the absorption of the gas, it may, perhaps, be suggested that possibly a stimulating action is exercised on the protoplasm, resulting in increased photosynthesis and respiratory activity, a preponderance of the latter process explaining the absence of increase in weight.

Effect of Carbon Dioxide on Geotropism.

The fact that roots placed horizontally in boiled water do not respond to geotropic stimulus, owing to the absence of oxygen, has been known for some time. A similar failure to respond results when the roots are placed in hydrogen or other indifferent gas. That the stimulus is perceived in each case is clearly proved by the fact that, if the plant be removed from the boiled water or gas and placed vertically in air, the root-tip executes a movement out of the vertical in the direction of the previous stimulus. Czapek's ammoniacal silver nitrate and guaiacum reactions (10) seem to offer a means of demonstrating such a perception, whether the stimulus be followed by curvature or no.

In view of the fact that carbon dioxide is a protoplasmic poison, and in small doses acts as a stimulant to streaming and to growth, it occurred to us that an investigation of its action on geotropic curvatures might be not without interest. With this object, experiments were set up as follows :—

Method I.—Peas, whose radicles had emerged to a length of about 3 cm., were fixed horizontally on a strip of cork in a glass vessel, through which gases could be passed. Through one such vessel carbon dioxide was passed for various lengths of time, a second vessel being employed as an air control. While horizontal no bending took place in either set. The peas were then placed with their roots in a vertical position in air, care being taken to keep the atmosphere saturated with water vapour, and the first appearance of curvature was noted. When the stimulus was allowed to act on the horizontally placed roots for 15 minutes, no appreciable difference could be

observed between the rates of curvature in the two sets when both were replaced in air at the close of the application of the stimulus. When, however, the stimulus acted for 20 minutes, in every case the curvature commenced appreciably sooner in the plants treated with carbon dioxide. The difference between the excess of bending in the plants stimulated in carbon dioxide for 15 and 20 minutes over that in their respective air controls is, perhaps, to be attributed to the slow penetration of the cells by the gas.

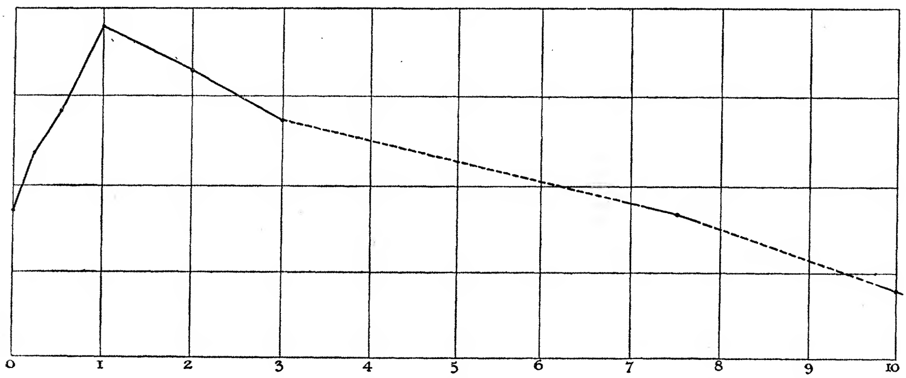
After 25 minutes' stimulation, the excess of curvature in the plants treated with carbon dioxide over that of the air-control plants was distinctly observable, though it was markedly less than in the plants treated for 20 minutes. It is probable that, during the additional 5 minutes, the carbon dioxide penetrates the cells in sufficient quantity to produce a solution in the cell-sap of supra-optimal strength, whereas in 20 minutes the optimal strength may be just attained.

Method II.—The above results seemed to be of sufficient interest to warrant a continuation of the investigation under more rigid experimental conditions. Plants, after stimulation in a horizontal position, were placed on a klinostat in such a manner that the axis of the root was parallel to the axis of rotation. The klinostat performed one revolution in 19 minutes—a sufficiently slow rate, according to Mr. Francis Darwin's (11) researches, to allow movement of the starch grains from side to side of the cell. Under these conditions, it was again found that no appreciable difference between the plants stimulated for 15 minutes in carbon dioxide and in air could be detected. After 20 minutes' stimulation, however, as before, the curvature of the roots treated with carbon dioxide was much more rapid.

Method III.—The plants were at once immersed in pure carbon dioxide, and were subsequently transferred to the klinostat. After stimulation for 20 minutes in carbon dioxide, the subsequent geotropic response set in distinctly earlier than in the air-control plants.

Method IV.—Mixtures of different percentages of carbon dioxide in air were employed with a view to determining the optimal partial pressure for geotropic response. The mixed gases were continuously passed over the peas, which were kept in a horizontal position throughout the experiment. The amount of curvature was recorded after stated intervals. By this means comparison is instituted between the curvatures due to continuous geotropic stimulus in atmospheres exerting different partial pressures of carbon dioxide. In the preceding methods the curvature was that following stimulation for limited and short periods, during which alone the carbon dioxide was allowed to act.

In order to record the curvatures observed, the following notation is adopted. The least appreciable curvature is denoted by *b*− ; *b*, *b*+, and *b*+ +, being used to express successively greater degrees of curvature. By reading against squared paper it has been found possible very accurately to allot values to these letters. If *b*− be equivalent to a value 3, then *b* is 4, *b*+ is 5, and *b*+ + is 7. By employing the values thus obtained, a curve graphically expressing our results with different partial pressures has been plotted.



Curve showing Amount of Curvature in different Partial Pressures of Carbon Dioxide. Ordinates express curvature and abscissæ percentages of carbon dioxide.

Series A.—Mixtures employed: Air; 1 per cent. CO_2 in air; 10 per cent. CO_2 in air. The response in air was more rapid than in 10 per cent. CO_2, but distinctly less rapid than in 1 per cent. CO_2 (see Table I).

Series B.—Mixtures employed: Air; 3 per cent. CO_2 in air; $7\frac{1}{2}$ per cent. CO_2 in air. Curvature was effected slightly more quickly in 3 per cent. CO_2 than in air, while but little difference could be observed between the plants in air and in $7\frac{1}{2}$ per cent. CO_2 (see Table II).

Series C.—Mixtures employed: Air; 1 per cent. CO_2 in air; 3 per cent. CO_2 in air. The curvature was first apparent in 1 per cent., while in 3 per cent. it appeared earlier than in air (see Table III).

Series D.—Mixtures employed: Air free from CO_2; Air; 2 per cent. CO_2. Curvature first set in in 2 per cent. CO_2, that in air being more marked than in air free from CO_2 (see Table IV).

Series E.—Mixtures employed: 1 per cent. CO_2; 2 per cent. CO_2. The first bending occurred in 1 per cent. CO_2 (see Table V).

Series F.—Mixtures employed: 0·2 per cent. CO_2; 0·5 per cent. CO_2; 1 per cent. CO_2. The earlier bendings were most vigorous in 1 per cent.,

those in 0·5 per cent. and in 0·2 per cent. following in decreasing order (see Table VI).

A few words of explanation may be added to the preceding account of our results. Attention was principally focussed upon the first appearance and relative magnitude of the earlier curvatures. In addition it should be noted that in CO_2 free air, in air itself, in 0·2, 0·5, 1, 2, and 3 per cent. CO_2, all the roots finally exhibited very large curvatures, while in $7\frac{1}{2}$ per cent., and in 10 per cent., the actual power of response seemed to be not only delayed, but actually diminished to a very large degree.

Throughout the experiments the range of temperature did not exceed about one degree Centigrade, namely, from 15° to 16°, and in every case any such small range was exactly similar for each set of peas used in comparison.

A few representative tables have been selected, and are given below:—

Table I.

Per cent. CO_2.	No. of peas.	Curvature after 45 mins.	1 hr. 15 mins.	1 hr. 45 mins.	2 hrs. 15 mins.	2 hrs. 45 mins.
Air ……	6	0	3*b*−	*b*+, *b*, 2*b*−	*b*+, *b*, 3*b*−	2*b*+ +, 2*b*+, *b*
1………	6	3*b*−	2*b*, 3*b*−	2*b*+, *b*, 3*b*−	2*b*+, 2*b*, 3*b*−	*b*+ +, 2*b*+, 2*b*
10………	6	0	0	2*b*−	3*b*−	3*b*, 2*b*−

Table II.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 30 mins.	2 hrs.	3 hrs.
Air ……	8	0	2*b*−	3*b*−	2*b*+, *b*, 4*b*−	*b*+ +, 4*b*+, 2*b*, *b*−
3 ………	8	0	3*b*−	3*b*−, *b*	*b*+, 2*b*, 3*b*−	3*b*+ +, *b*+, 2*b*, *b*−
$7\frac{1}{2}$………	8	0	2*b*−	2*b*−	*b*, *b*−	3*b*, 2*b*−

Table III.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 30 mins.	2 hrs.	3 hrs.
Air ……	6	*b*−	2*b*	3*b*, *b*−	2*b*+, *b*, 2*b*−	4*b*+, *b*, *b*−
1 ………	5	2*b*−	2*b*, 2*b*−	2*b*+, 2*b*, *b*−	3*b*+ +, *b*+, *b*	4*b*+ +, *b*+
3 ………	6	*b*−	*b*, 3*b*−	*b*+, 3*b*, 2*b*−	*b*+ +, 4*b*+, *b*−	2*b*+ +, 4*b*+

Table IV.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 45 mins.	2 hrs. 15 mins.
0...........	8	3*b* –	5*b* –	3*b*, 3*b* –	*b* +, 2*b*, 3*b* –
Air.........	8	0	*b*, 3*b* –	*b* +, 2*b*, 5*b* –	3*b* +, 2*b*, 3*b* –
2...........	8	*b*, *b* –	2*b*, 4*b* –	2*b* +, 3*b*, 3*b* –	*b* + +, 2*b* +, 4*b*

Table V.

Per cent. CO_2.	No. of peas.	Curvature after 1 hr. 15 mins.	1 hr. 45 mins.	2 hrs. 30 mins.	3 hrs.
1...........	12	2*b*, 6*b* –	8*b* +, 2*b*, 2*b* –	*b* + +, 8*b* +, 3*b*	3*b* + +, 8*b* +, *b*
2...........	12	8*b* –	6*b* +, 2*b*, 4*b* –	9*b* +, 2*b*, *b* –	*b* + +, 10*b* +, *b* –

Table VI.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 30 mins.	2 hrs.	2 hrs. 30 mins.
0·2	12	0	4*b* –	3*b*, 6*b* –	*b* +, 5*b*, 5*b* –	5*b* +, 6*b*
0·5	12	0	6*b* –	5*b*, 4*b* –	3*b* +, 5*b*, 3*b* –	*b* + +, 6*b* +, 3*b*, *b* –
1·0	12	0	*b*, 7*b* –	2*b* +, 6*b*, 3*b* –	8*b* +, 3*b* –	*b* + +, 9*b* +, *b*, *b* –

In considering the mode of action of carbon dioxide several possibilities present themselves. It is conceivable that increase of the partial pressure of carbon dioxide above a certain point, by the mere presence of an increased number of molecules of this substance, may interfere with the liberation of more carbon dioxide in the ordinary respiratory function, so depressing the activity of the cell. On the other hand, or in addition to this mode, the carbon dioxide may exert a direct action on the protoplasm itself. Although this seems to have been the generally accepted explanation, it appears to afford but a very imperfect picture of the true course of events. Recent work on the action of poisons suggests with considerable force that in many cases dissociation of the molecule precedes the manifestation of physiological effect. Walker (12) has shown that when carbon dioxide is dissolved in water, a solution containing unchanged CO_2, H_2CO_3 and dissociated H_2CO_3 (H^+ and HCO_3^-) is produced, the proportion of the dissociated substance depending on the strength of solution.

It is not improbable that the dissociated substance is principally concerned in the causation of the physiological effects described above. The manifestation of these results may be due to the action of the H, or of the HCO_3 ions. (The possibility of direct action of H_2CO_3 being of the same order as that of the unchanged CO_2.) It is hoped shortly to publish the results of experiments undertaken with a view to the elucidation of this at present obscure problem.

Whether the action take place by union with the protoplasm itself, or by combination with some metabolite, thereby putting this latter substance out of the field of physiological action, remains to be determined.

It must be borne in mind that the external partial pressure of carbon dioxide alone is known, whereas it is the partial pressure within the pea that determines the strength of solution and the amount of ionisation. It is true that the external partial pressure to a very considerable extent determines the internal pressure, but in addition to this is the carbon dioxide constantly evolved in respiration of the cells. At a certain stage the carbon dioxide penetrating from without, together with that evolved by the cells, will create an internal partial pressure equal to the external one. But as respiration proceeds the partial pressure within the pea will exceed that outside, and a slightly higher internal pressure will be maintained as long as respiration continues. Hence, what has been determined by the above experiments is the partial pressure of carbon dioxide in the external atmosphere sufficient to produce during the time of experiment the optimum strength of solution in the cell sap.

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Preliminary Note on the Occurrence of Microsporangia in Organic Connection with the Foliage of Lyginodendron.

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(Plate 6.)

Any certain knowledge at present possessed of the fructification of the *Pteridospermeæ** is restricted to the female organ or seed. Suggestions have been made that the microsporangia of *Lyginodendron Oldhamium*, the *Sphenopteris Höninghausi* of Brongniart, might be found in *Telangium Scotti*,† but the evidence for this was chiefly its association with fragments of *Lyginodendron Oldhamium*. From the structure of *Telangium Scotti*, I am satisfied that it cannot be the microsporangia of *Sphenopteris Höninghausi*, and in any case the organic connection was not demonstrated.

Among some specimens from the 10-foot ironstone measures (Westphalian, series), Coseley, near Dudley, sent me for examination by Mr. H. W. Hughes, F.G.S., were a number of examples of *Sphenopteris Höninghausi* preserved in small nodules.

Many of these were fragments of barren pinnæ, but a few showed a fructification referable to *Crossotheca*, Zeiller,‡ in organic connection with barren foliage of *Sphenopteris Höninghausi*, while other specimens consisted of fertile pinnæ or portions of pinnæ unassociated with any barren pinnules. Their identity with the fertile pinnules found in connection with sterile ones, leaves no doubt as to their also belonging to *Sphenopteris Höninghausi*.

I do not propose to enter into a detailed account of the structure of the

* Oliver and Scott, "On the Structure of the Palæozoic Seed *Lagenostoma Lomaxi*, etc.," 'Phil. Trans.,' ser. B, vol. 197, p. 239, 1904.

† M. Benson, *Telangium Scotti*, a new species of *Telangium* (*Calymmatotheca*), showing structure, 'Ann. of Botany,' vol. 18, p. 161, Pl. 11, 1904.

‡ *Crossotheca*, Zeiller, 'Ann. d. Sc. Nat., 6e sér., Bot.,' vol. 16, p. 180, Pl. 9, figs. 1—9, Aug., 1883, "Flore foss. Bassin houiller d. Valenciennes," p. 33, fig. 21, 1888.